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Vertebrate Left-Right Development

Minireview

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Significant biological asymmetries range from the differentiation of sides within a single cell to the establishment of embryonic pattern across large fields of cells in multicellular organisms. One of the most intriguing biological asymmetries is left–right asymmetry in vertebrates. All vertebrates display a number of structures that are asymmetric along the left–right geometric body axis. The consistent alignment, or handedness, of left–right asymmetric organs relative to the dorsoventral and anteroposterior axes is evolutionarily conserved. For example, the heart tube loops to the right in all chordates. Alterations in laterality of left–right asymmetric organs has severe clinical consequences in humans (Burn, 1991). Left–right brain asymmetries in both structure and function are a focus of intense research (Davidson and Hugdahl, 1995) and popular speculation.

The development of left–right asymmetries presents fundamental problems that are not encountered in studies of other embryonic axes (Figure 1). In the best-characterized model system for vertebrate embryonic axis formation (Xenopus laevis), eggs are cylindrically symmetrical before fertilization. A dorsal midline can arise at any line between the animal and vegetal poles, indicating that all points along the circumference have equivalent developmental potential. Once the dorsal midline has been determined, by a cytoplasmic rotation in the first cell cycle after fertilization, the dorsoventral and anteroposterior (a consequence of the animal–vegetal axis) axes of the embryo are by definition in register with each other (Gerhart et al., 1989). Establishing a midline divides the embryo into left and right halves in a trivial sense. However, because all points along the circumference had equal developmental potential before the assignment of a dorsal midline (indicated by Xs in Figure 1A), the left and right sides of the embryo should be bilaterally symmetric. Positions at a given distance from the midline (Figure 1B; distances from the midline are indicated by B, C, D, and E) should be identical but mirror images of each other (for example, at the C and C′ positions). However, in all vertebrates studied to date, this is not the case. Not only are there left–right asymmetries, but these asymmetries are consistently aligned with respect to the dorsoventral and anteroposterior axes. Therefore, when considering the development of left–right handedness, in addition to pattern formation mechanisms that are common to all embryonic axes, there is the unique problem of consistently aligning the left–right asymmetries with the other embryonic axes.

The generation of the vertebrate left–right axis requires both a mechanism that generates asymmetry and a mechanism that consistently aligns left–right asymmetry with the other embryonic axes. Elimination of the second mechanism, by genetic mutation or experimental manipulation, is termed the randomization of left–right orientation: the transition from bilateral symmetry to left–right asymmetry still occurs, but the orientation relative to the dorsoventral and anteroposterior body axes is stochastically determined (Brown and Wolpert, 1990; Wilhelmi, 1921). Any model for how consistent left–right handedness is generated must explain both aspects of left–right development.

Genetic Alterations of Left-Right Development

There are two well-described mutants in mice that alter left–right development with few other embryonic defects. The *iv/iv* mutant mouse has randomized left–right orientation, displaying a full array of reversals and isomerisms (mirror images) in the heart and the viscera (Seo et al., 1992). The *iv* locus has been mapped to chromosome 12 (McGrath et al., 1992). Homozygotes of *inv*, a serendipitously obtained insertional mutation mapped to chromosome 4, show close to 100% reversal rates of the heart,

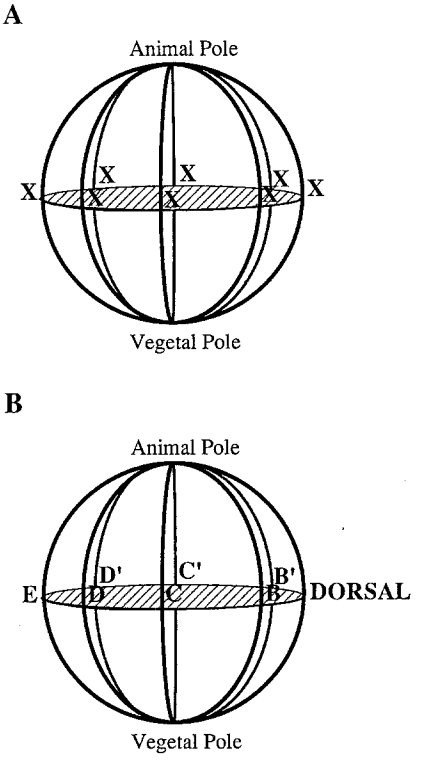

Figure 1. The Fundamental Problem of Bilateral Symmetry and Consistent Left–Right Asymmetry

(A) In X. laevis eggs before the assignment of a dorsal midline, each line between the animal and vegetal poles has equal developmental potential (as indicated by the Xs).

(B) After establishment of the dorsal midline, both bilateral symmetries (an equal distance from the midline; C is a mirror image of C′) and consistent left–right asymmetries (C is not equal to C′) emerge. An underlying asymmetry might precede the establishment of the midline and interact with the superimposed midline. For example, the cytoplasmic rotation that establishes the dorsal midline during the first cell cycle might be consistently asymmetric along the prospective left–right axis.

viscera, and body/tail orientation (Yokoyama et al., 1993). This phenotype is quite different from the randomization of left–right orientation obtained in the embryological manipulations described below or in *iv/iv* mice and has been difficult to incorporate into models (Klar, 1994).

Two other mouse mutants have been described that might have defects in left–right development. *Mgat1* (on chromosome 11) encodes N-acetylglucosaminyl transferase I, a key enzyme in biosynthesis of complex N-linked oligosaccharides on cell surface and extracellular proteins. Homozygotes of a *Mgat1* knockout die by midgestation and show significant neural defects, including convoluted neural epithelium, and vascularization defects. Some of the homozygous mutants have reversed heart and inverted tail position, although the frequencies of these phenotypes are not reported (Metzler et al., 1994) and cardiac reversals were not reported in another *Mgat1* knockout mouse (Ioffe and Stanley, 1994). The insertional mutation fused toes (*Ft*) shows a dominant (heterozygous) phenotype of fused toes, proposed to be a defect in the developmental regulation of cell death. Homozygotes die by embryonic day 10 and have greatly reduced telencephalon and mesencephalon. Approximately half of the homozygotes that were assayed had reversed orientation tail coiling; other left–right morphologies were not reported (van der Hoeven et al., 1994). Given the complex embryological defects in each of these mutants, it is likely that alterations in left–right development are secondary defects, perhaps reflecting the linkage of dorsoanterior and left–right development (Danos and Yost, 1995).

In humans, several syndromes display laterality defects (Burn, 1991). Kartagener's syndrome is an autosomal recessive defect in dynein arms (Afzelius, 1976). Recently, a laterality mutation has been mapped to the X chromosome in humans (Casey et al., 1993). The molecular characterization of left–right signaling pathways in model systems should eventually increase our understanding of human laterality syndromes and provide candidate genes to test against autosomal recessive, autosomal dominant, and X-linked inheritance cases.

Embryology and Molecular Biology of Left–Right Orientation

Over many years, several embryological methods have been shown to alter left–right development (see below; reviewed by Brown and Wolpert, 1990). The effects of most of these treatments fall into one of two categories: either preventing the morphological transition from symmetry to asymmetry or randomizing the orientation or handedness of left–right asymmetry. Since the left–right body axis is defined relative to the dorsoventral and anteroposterior body axes, it appears reasonable that common developmental mechanisms and shared signaling molecules are involved in regulating all three body axes in a coordinate manner. In very early embryos, treatments that randomize left–right orientation also perturb development of the other embryonic axes, truncating dorsoanterior structures such as the forebrain and midbrain. Partial inhibition of cytoplasmic rotation that establishes the embryonic dorsoanterior axes results in diminished dorsoanterior development and correspondingly randomizes cardiac left–right orientation. Ectopic expression of *Xwnt8*, a member of the *Wnt* family of cell-to-cell signaling molecules, in dorsal cells before gastrulation gives an identical phenotype. These results indicate that normal dorsal midline cells, which form the embryonic organizer and the notochord, are necessary for normal cardiac left–right orientation (Danos and Yost, 1995).

Other embryological treatments, including perturbations of extracellular matrix (ECM) in Xenopus gastrula (Yost, 1992) or stimulation of the α1A adrenergic receptor subtype in rat neurula-stage embryos (Fujinaga et al., 1994), result in randomization of cardiac asymmetry without apparent effects on dorsoventral and anteroposterior development. Altered gap junction communication has also been implicated as a cause of cardiac laterality defects in humans (Britzcunningham et al., 1995). These results suggest that cell–ECM or cell–cell interactions are involved in left–right development.

Hensen's node (HN) of chick embryos is analogous to the organizer in frog embryos and is thought to orchestrate anteroposterior body axis formation and dorsoventral axis formation in the neural tube by cell-to-cell signaling. Work reported in this issue of *Cell* has advanced our understanding of left–right development to the molecular level (Levin et al., 1995). Four RNAs are asymmetrically distributed along the left–right axis in chick embryos during gastrulation and neurula stages, predominantly in HN or near the midline: activin receptor IIa (*cAct-RIIa*), *Sonic hedgehog* (*Shh*), the transcription factor *HFN3β*, and the chicken *nodal*-related gene *cNR-1*. *cAct-RIIa* is more abundant in the primitive ridge on the right side of the primitive streak and then exclusively expressed in the ectoderm on the right side of HN. *Shh* is expressed symmetrically in early HN (stage 4) and then expressed only in the left side of HN, in the ectoderm, from stages 4^+ through 7. *HFN3β* expression is only briefly asymmetric at an early stage (stage 4^-) in a small part of the left side of the primitive ridge, perhaps induced by the larger amount of *Shh* in the left side of HN. Ectodermal cells in the left side (expressing *Shh*) and in the right side (expressing *cAct-RIIa*) of HN ingress during gastrulation and interdigitate to form the notochord at the embryonic midline. *cAct-RIIa*, *Shh*, and *HNF3β* continue to be expressed in notochord cells anterior to HN. *cNR-1* is symmetrically expressed early, then disappears, and reappears at stage 7 just on the left side, lateral and anterior to the *Shh*-expressing region. Later, the expression of *cNR-1* is expanded in the left lateral plate mesoderm, which includes heart progenitor cells (Levin et al., 1995).

There are several remarkable aspects of these expression patterns. The genes expressed asymmetrically along the left–right axis are members of gene families that have been implicated in cell signaling and in the regulation of dorsoventral and anteroposterior axes. The familiarity of developmental biologists with these signaling molecules allows ready manipulation of the proposed pathways for left–right development. The asymmetric expression patterns are transient, occurring during a relatively narrow period during gastrulation, and are either preceded or followed by symmetric expression patterns. Both sides of

the embryo display asymmetric expression patterns: *cAct-RIIa* on the right side and the others on the left. Finally, the temporal order and specific juxtapositions of the expression patterns in the embryo have allowed Levin et al. (1995) to postulate a sequential order of signaling, beginning with asymmetric expression of the activin receptor and ending in abundant expression of *cNR-1* in the lateral plate that will give rise to primordia that display morphological asymmetry.

Levin et al. (1995) tested molecular signaling pathways in left–right development in chick embryos by ectopically expressing some of the molecules in the implicated pathway and assessing both the expression patterns of the other asymmetrically expressed RNAs and cardiac left–right orientation. Implanting an activin-emitting bead on the left side of the node gives ectopic expression of *cAct-RIIa* and loss of *Shh* on the left side of HN as well as reduction of *cAct-RIIa* in the lateral plate mesoderm on the right side and loss of *cNR-1* expression on the left side. Subsequent cardiac left–right orientation is randomized. Implanting *Shh*-producing cells on the right side of HN results in ectopic *cNR-1* expression on the right side and randomization of cardiac left–right orientation. Implanting *Shh*-producing cells on the left side has no effect on normal *Shh* and *cNR-1* expression patterns and does not alter normal cardiac left–right development. From these results, Levin et al. (1995) propose that an activin-like activity is present on the right side, activating *cAct-RIIa* and suppressing *Shh* on the right. Absence of the activin-like activity on the left side allows asymmetric *Shh* expression in the left HN, which then induces *cNR-1* on the left, eventually leading to asymmetric heart development.

In the Beginning?

Cell-to-cell signaling is clearly involved in the regulation of left–right development. At some point in early development, an initial handedness or left–right orientation must be established with respect to the dorsoventral and anteroposterior axes (Figure 1) (Danos and Yost, 1995). We do not know how the initial left–right orientation is established or what the initial asymmetric cell-to-cell signal is. Levin et al. (1995) postulate that an activin-like activity on the right side of the embryo or a follistatin-like activity that counteracts activin-like activity on the left side of the embryo should precede the molecular asymmetries they have discovered in chick, but such asymmetric distributions have not yet been described. In Xenopus, vegetal endoderm cells are thought to initiate formation of the mesoderm and the organizer; the inductive capacity of these cells diminishes earlier on the left than on the right (Nieuwkoop et al., 1985). This left–right orientation might be due to a left–right asymmetry in the microtubule-dependent cytoplasmic rotation that establishes the dorsoventral axis during the first cell cycle (Yost, 1991). Directed segregation of nonequivalent chromatids of a specific chromosome to daughter cells in early embryos has been proposed as a mechanism for establishing left–right asymmetry, but the model does not explain how non-equivalent chromatid segregation can be aligned with the embryonic axes to give consistent left–right orientation (Klar, 1994). Morphological asymmetries that might be responses to asymmetric induction of the organizer or HN are evident in the chick (Cooke, 1995) and in the propagation of cleavage patterns in Xenopus (Nieuwkoop et al., 1985). Taking a cue from development in the worm (Priess, 1994), perhaps the earliest asymmetries in vertebrate embryos will prove to be unequal distributions of intracellular components that then modulate left–right cell-to-cell signaling.

In the End: Morphogenesis of Asymmetry

To understand the consequences of molecular signaling pathways, we must discover not only the mechanisms by which signaling molecules become asymmetrically distributed, but the mechanisms by which cells interpret asymmetrically expressed signals to form asymmetric organs. In normal animals, the left–right orientation of various asymmetric structures is coordinated; i.e., the heart loops to the right and the stomach bends to the left. This coordination suggests that each organ primordium responds to a common set of left–right orientation signals. In embryos in which primordium–ECM interaction is experimentally altered (Yost, 1992) or in *iv/iv* mutants (McGrath et al., 1992; Seo et al., 1992), the left–right orientation of organs is randomized, such that the orientation of each organ is statistically independent of the orientation of adjacent organs in the same individual (termed heterotaxia). However, left–right asymmetries appear to be generated by distinct morphological mechanisms in different organ primordia. For example, the generation of cardiac asymmetry by the looping of the predominantly bilaterally symmetric tube is likely to be a different morphogenetic process than the orientation and alignment of primordia that form liver, gallbladder, and pancreas or the generation of morphological asymmetry in the amphibian brain. From this, one could postulate that different primordia receive the same underlying asymmetric molecular signal, but that each primordium interprets the asymmetric signals to its own end. The underlying assumption is that signals that establish left–right orientation in organ primordia are separate from signals that establish the identities of organ primordia.

Several morphogenetic mechanisms by which the cardiac tube undergoes the transition from symmetry to asymmetry have been postulated and to some extent tested. One possibility is that more cells accumulate on one side of the tube and cause the tube to bend outward. Differences in cell number could arise by differential cell division, cell death, or early differences in the recruitment of cells into cardiac primordia. In the chick, more progenitors contribute to the right side of the cardiac tube, and it loops out to the right (Stalsberg, 1969). Alternatively, cardiac tube looping could be driven by cell shape changes or differential contraction of the cytoskeleton. Broad application of cytochalasin B, an inhibitor of actin polymerization, blocks looping of the cardiac tube in chick embryos; localized application of cytochalasin B can reverse the direction of cardiac tube bending (Itasaki et al., 1991). Inhibition of proteoglycan synthesis during early neurula stages in Xenopus, long before the cardiac tube is formed, blocks cardiac tube looping. Since proteoglycans are predominantly cell surface or extracellular molecules, these results imply that early cell signaling is necessary for cardiac tube looping (Yost, 1990).

In summary, we have a long way to go before we can describe the molecular pathways that establish left–right orientation in the vertebrate, but the focus is now on signaling peptides and transient cell interactions. Ectopic expression in embryos of members of three important signaling peptide families, *Wnt* (Danos and Yost, 1995), activin, and *Shh* (Levin et al., 1995), result in randomization of left–right asymmetries. The sequential signaling pathway discovered by Levin et al. (1995) provides a starting point from which to work backward in developmental time to the primary embryonic left–right orientation and forward in developmental time to the cellular and molecular events that regulate the left–right morphogenesis of many important organ systems.

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